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Review

Biochemistry and physiology of mitochondrial ion channels involved in cardioprotection

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ABSTRACT

Over the past decades there has been considerable progress in understanding the multifunctional roles of mitochondrial ion channels in metabolism, energy transduction, ion transport, signaling, and cell death. Recent data have suggested that some of these channels function under physiological condition, and others may be activated in response to pathological insults and play a key role in cytoprotection. This review outlines our current understanding of the molecular identity and pathophysiological roles of the mitochondrial ion channels in the heart with particular emphasis on cardioprotection against ischemia/reperfusion injury, and future research on mitochondrial ion channels.

1. Introduction

Mitochondria play an important role in energy metabolism within the cell. In addition, the organelles are also involved in regulation of cell death and survival, a crucial determinant of apoptosis or necrosis. However, the physiological roles of mitochondrial ion channels on inner membrane are largely unknown. For example, K^+-selective and/or anion-selective pores are believed to operate to regulate mitochondrial volume [1], but it is difficult to achieve in vivo confirmation of this function [2]. Mitochondrial swelling and shrinkage have been proposed to modulate the rate of substrate oxidation under normoxic conditions [3] and could be an important factor in determining the extent of ischemia/reperfusion injury [4]. At a minimum, energy dissipation due to channel opening will increase the flux through the electron-transport chain, and if this effect is uncompensated by increased substrate production, then net oxidation of the matrix, as well as a change in reactive oxygen species (ROS) production, will occur. Thus one potential physiological role of mitochondrial ion channels may be redox regulation, which is an important intracellular signaling mechanism influencing transcription, translation, phosphorylation cascade, and cell death.

In the cardiovascular system mitochondria are target organelles of ischemic insults as well as effectors for cardioprotection. As pointed out by Jennings and Ganote 30 years ago [5], the heart is strictly aerobic and consequently vulnerable to a decrease in oxygen supply. Therefore, myocardial ischemia causes immediate and deep mitochondrial derangements. These include cessation of ATP synthesis, inhibition of respiration, and depolarization of mitochondrial membrane ($\Delta\Psi m$). This is accompanied by cellular changes, especially an increase in Ca^{2+} and phosphate during ischemia, and large increases in ROS originating from the respiratory chain during reperfusion [6]. Also from the therapeutic perspective, with reference to ischemia/reperfusion injury in the heart and brain, it is important to note that mitochondrial ion channels especially play a key role. An increase in mitochondrial K^+ flux, for example, in hearts treated with K^+ channel opener compounds activating mitochondrial ATP-sensitive K^+ ($mitoK_{ATP}$) or Ca^{2+}-activated K^+ channels ($mitoK_{Ca}$), has been found to significantly decrease infarct size and improve functional recovery after ischemia/reperfusion. On the other hand, blocking mitochondrial ion channels such as the mitochondrial permeability transition pore (mPTP) can prevent the loss of mitochondrial function that leads to necrotic or apoptotic cell death. Since cardioprotection

Abbreviations: ROS, reactive oxygen species; $\Delta\Psi m$, mitochondrial membrane potential; $mitoK_{ATP}$ channel, mitochondrial ATP-sensitive K^+ channel; $mitoK_{Ca}$ channel, mitochondrial Ca^{2+}-activated K^+ channel; mPTP, mitochondrial permeability transition pore; RuR, ruthenium red; $sarcK_{ATP}$ channel, sarcolemmal ATP-sensitive K^+ channel; VDAC, voltage-dependent anion channel; ANT, adenine nucleotide translocase; CypD, cyclophilin D

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involves the activation of mitoK$_{ATP}$ or mitoK$_{Ca}$ channels and a decrease in mPTP opening, it is reasonable to hypothesize that these two phenomena are part of the same signaling pathway. Indeed, this connection has been demonstrated in a previous report [7].

In this review we describe our current understanding of the molecular identity and pharmacological properties of important four mitochondrial ion channels conferring cardioprotection (see the property of these ion channels in Table 1).

2. The mitochondrial Ca^{2+} uniporter

Mitochondrial Ca^{2+} uptake has been recognized for more than 50 years, yet the protein mediating this essential transport process has still not been clarified. However, the mitochondrial Ca^{2+} uniporter has perhaps the most well-understood transporter in physiology. The Ca^{2+} uniporter is a relatively fast mechanism and a channel, which transports Ca^{2+}, Sr^{2+}, Mn^{2+}, Ba^{2+}, but not Mg^{2+} with different selectivity and very low affinity [8]. Because of the low number of uniporters found per mg protein (around 0.001 nmol/mg protein reported [9]) and the large value of the estimated Vmax [$\sim$1200 nmol Ca^{2+}/(mg min)] the turnover of Ca^{2+} per site can be calculated to be about 2×10^4 Ca^{2+}/(site s) [10], in the range of a fast gated pore. Studies performed on isolated mitochondria allowed the identification of some regulatory molecules acting on Ca^{2+} uniporter, in particular the most effective inhibitor is hexavalent cation ruthenium red (RuR) [11]. This RuR-sensitive Ca^{2+} uptake pathway is the primary route for Ca^{2+} entry into the mitochondrial matrix, and there is strong evidence that an increase in matrix Ca^{2+} is important for stimulating oxidative phosphorylation at several sites, including the Ca^{2+}-sensitive dehydrogenases of the Krebs cycle [11] and one or more sites in the electron-transport chain [12]. This uniporter is modulated by aliphatic polyamines, such as spermine and aminoglycosides, and by the adenine nucleotides, in the order of effectiveness ATP > ADP > AMP (whereas the nucleoside adenosine is ineffective) [13] as well as several plant-derived flavonoids [14]. Recently, an inwardly rectifying, highly Ca^{2+} selective, low conductance ion channel that is compatible with mitochondrial Ca^{2+} uniporter was recorded in a patch-clamp study of intact heart mitoplasts [15]. However, the molecular structure of this channel has not yet been determined despite a number of attempts to identify Ca^{2+} uniporter protein over years. Trenker et al. suggested that the uncoupling proteins UCP2 and UCP3 were promising candidates for enabling Ca^{2+} uptake in mitochondria since isolated liver mitochondria from UCP2 knockout mice showed no RuR-sensitive Ca^{2+} uniporter activity [16]. On the opposite, in new data submitted by Brookes et al. there was no effect of UCP2 or UCP3 knockout on either Ca^{2+} uptake or membrane potential depolarization in mitochondria from liver, skeletal muscle, heart, or kidney [17].

As described below, the inhibition of Ca^{2+} influx to matrix through mitochondrial Ca^{2+} uniporter by mitochondrial K^+ cannel opening results in decrease of mitochondrial Ca^{2+} overload. Mitochondrial Ca^{2+} loading is associated with cell injury although the relative contributions of cytoplasmic vs. mitochondrial Ca^{2+} overload are often difficult to assess individually due to their interdependence, and there is not always a clear correlation between mitochondrial Ca^{2+} and survival [18].

3. Mitochondrial K^+ channel and cardioprotection

Some 25 years ago Lamping et al. showed that pharmacological agents capable of opening K^+ channels have cardioprotective effect against ischemia/reperfusion injury [19]. Earlier studies naturally supposed that the target of these compounds was the sarcolemmal ATP-sensitive K^+ (sarcK$_{ATP}$) channel. However, a new door was opened in 1991, with the discovery of mitoK$_{ATP}$ channels in the liver mitochondrial inner membrane [20]. This finding was bolstered by evidence that a variety of K^+ channel openers and inhibitors influenced mitochondrial function [1,21–23], resulting in the establishment of a link between the mitoK$_{ATP}$ channel and cardioprotection against ischemia/reperfusion injury in intact hearts [23] and isolated myocytes [24]. Therefore, the focus has shifted recently to the mitochondria as the primary target of these agents.

3.1. Mechanism of cardioprotection by mitochondrial K^+ channel

One of the major physiological features of mitochondria is the generation of a large transmembrane potential across the mitochondrial inner membrane. This is a direct consequence of the biochemical reactions that constitute the respiratory chain. Thus, substrates supplied to mitochondria such as pyruvate, products of β-oxidation of fatty acids, and some amino acids enter the TCA cycle and maintain the reduced state of the NADH/NAD$^+$ and FADH$_2$/FAD couples. These substances supply electrons to the respiratory chain, which eventually are transferred to oxygen. The process also transfers protons across the mitochondrial inner membrane, generating a proton gradient – a proton motive force that is largely expressed as $\Delta\Psi$m usually estimated as 180 mV negative to the cytosol. The Ca^{2+} uptake into mitochondria is driven primarily by this large negative electrical potential of the

Table 1
Mitochondrial ion channels involved in cardioprotection.

Location	Type	Conductance ($\sim$150 mM salt)	Putative role	Modulators or inhibitors
Inner membrane	Ca^{2+} uniporter	6 pS	Ca^{2+} uptake	Divalent ion Nucleotides Ruthenium red
Inner membrane	ATP-sensitive K^+ channel	9.7 pS	Cytoprotection against ischemic injury Volume regulation Apoptosis	ATP GTP Mg^{2+} Ca^{2+} palmitoyl CoA
Inner membrane	Ca^{2+}-activated K^+ channel	295 pS	Cytoprotection against ischemic injury Volume regulation	Ca^{2+} Change in membrane voltage Charybdotoxin
Inner membrane	Permeability transition pore	0.3–1.3 nS	Necrosis Apoptosis	Cyclosporin A pH thiols Ca^{2+} Bax ANT inhibition

ANT, adenine nucleotide translocase.

matrix [10]. Therefore, partial depolarization of $\Delta\Psi m$ through opening of mitochondrial K^+ channels reduces the driving force for Ca^{2+} influx through the Ca^{2+} uniporter, which results in the prevention of mitochondrial Ca^{2+} overload during ischemia. Ishida et al. have reported that in rat cardiomyocytes the selective mito-K_{ATP} channel opener diazoxide depolarizes the mitochondrial membrane and attenuates the mitochondrial Ca^{2+} overload experimentally evoked by ouabain [25]. Ouabain, a Na^+/K^+-ATPase inhibitor, impairs Na^+ extrusion and consequently prevents Ca^{2+} extrusion via Na^+/Ca^{2+} exchange. Elevation in cytosolic Ca^{2+} concentration eventually results in the accumulation of mitochondrial Ca^{2+}. Furthermore, we have recently demonstrated that K^+ influx via mitochondrial K^+ channels accelerates electron transfer by the respiratory chain and leads to net oxidation of mitochondria if uncompensated by electron donors, and attenuates mitochondrial Ca^{2+} overload with accompanying depolarization of the mitochondrial membrane [26].

3.2. Mitochondrial ATP-sensitive K^+ channel

In 1991, Inoue et al. [20] reported the identification of an ATP-sensitive K^+ channel in the mitochondria. They patch clamped mitoplasts (mitochondria stripped of their outer membranes) from rat liver and identified K^+ selective channels, sensitive to inhibition by ATP, 4-aminopyridine and glibenclamide. The conductance of these channels was lower than that of ssrcK_{ATP} channels (about 10 pS in 100 mM matrix K^+ and 33 mM cytosolic K^+). Subsequent studies by several groups have demonstrated that mitoK_{ATP} may be the key player in cardioprotection.

Garlid's group has used two different approaches to study the channel. In their first approach, a protein fraction was purified on DEAE-cellulose columns [21], and an ATP-binding affinity column [27]. The fraction was reported to contain two major proteins of 55 and 63 kDa in size; the larger protein was reported to bind to fluorescently labeled glibenclamide [27]. The protein fraction was then incorporated into liposomes containing a K^+-sensitive fluorescent marker [21,28]. The emission intensity of this marker increases when K^+ binds to it. Thus, the rate of fluorescence increase can be used to quantify K^+ transport into the proteoliposomes [28]. In their second approach, they assessed steady state matrix volume by measuring light scattering at 520 nm in the intact isolated mitochondria [29]. The opening of mitoK_{ATP} channel and influx of K^+ result in an increase in the osmotic pressure and water movement into the mitochondrial matrix, which would lead to an increase in the mitochondrial matrix volume [1]. Thus, changes in the matrix volume can be used as an indirect measure of the mitoK_{ATP} channel activity.

Our study group has taken a different approach to assay the function of mitochondrial K^+ channels [24–26,30]. This approach is based on the autofluorescence of mitochondrial flavoproteins and NADH, which increases with partial uncoupling generated by mitoK_{ATP} channel activation [31,32]. The advantage of this technique is that it can be used in intact cells. However, there are also several limitations with this technique. Since the mitochondrial redox potential is a balance between NADH production and oxidation, it does not represent the overall rate of oxidative phosphorylation of the cell.

O'Rourke et al. have indicated that pharmacological drugs such as diazoxide open mitoK_{ATP} channel, and 5-hydroxydecanoate has been used as a blocker of mitoK_{ATP} channel [24,32]. MitoK_{ATP} channel openers such as diazoxide, nicorandil, minoxidil and so on have been shown to be cardioprotective in all species examined [33]. The ability of these agents to open mitoK_{ATP} channels in their therapeutic dose range was described in 1996 [1]. Diazoxide was 1000 times more potent in opening of mitoK_{ATP} channel than in opening of sarcK_{ATP} channel, making diazoxide a valuable tool to determine whether cardioprotection was mediated by the activation of sarcK_{ATP} or mitoK_{ATP} channels.

The molecular structure of sarcK_{ATP} channel has been clarified by cloning of the inwardly rectifying K^+ channel subfamily Kir6.0 (Kir6.1, 48-kDa and Kir6.2, 44-kDa) and the receptors for sulfonylureas (SUR1, 177-kDa, SUR2A, 174-kDa, and SUR2B, 174-kDa) [34]. From a reconstitution study, this channel on cardiomyocytes has been suggested to comprise SUR2A and Kir6.2 [35]. Our functional study using Kir6.2-deficient mice has provided direct evidence that Kir6.2 forms the pore region of cardiac sarcK_{ATP} channels [36]. On the other hand, molecular identification of mitoK_{ATP} channel has not been achieved yet. Although the molecular structure of mito-K_{ATP} channel remains unclear, experiments using dominant negative gene transfer have indicated that neither Kir6.1 nor Kir6.2 is a functionally important part of the mitoK_{ATP} channel in intact cardiomyocytes [37]. In addition, the mitoK_{ATP} channel function, evaluated by flavoprotein oxidation, was preserved in cardiomyocytes of either Kir6.1-deficient [38] or Kir6.2-deficient mice [39]. However, K^+ influx activity has been observed when purified mitochondrial proteins in the molecular weight range of 50–60 kDa have been reconstituted into proteoliposomes [40], and a 54-kDa protein was tentatively identified as a component of mitoK_{ATP} channel [21]. The existence of SUR proteins in cardiac mitochondria is still controversial [22], whilst a protein of about 28 kDa was labeled by [^{125}I]-glibencamide [41]. Recently, some study has reported that mitoK_{ATP} channel is potentially composed of four mitochondrial proteins (mitochondrial ATP-binding cassette protein 1, phosphate carrier, adenine nucleotide translocator, and ATP synthase) and succinate dehydrogenase, and multiprotein complex [42].

3.3. Mitochondrial Ca^{2+}-activated K^+ channel

The second mitochondrial inner membrane K^+ channel identified in the mitochondrial inner membrane by direct patch-clamp method of mitoplast was the Ca^{2+}-activated K^+ channel having properties resembling the channel of the surface membrane. MitoK_{Ca} channel was detected in mitochondria from human glioma cells in 1999 [43] and cardiac cell mitochondria in 2002 [44]. Our previous work has suggested that, in a similar manner to that for the mitoK_{ATP} channel, K^+ influx via mitoK_{Ca} channels accelerates electron transfer by the respiratory chain and leads to net oxidation of mitochondria if uncompensated by electron donors, and attenuates mitochondrial Ca^{2+} overload with accompanying depolarization of $\Delta\Psi m$ [26,30]. Furthermore, previous study in our laboratory has also showed that opening of mitoK_{Ca} channels is modulated by PKA and confers cardioprotection against ischemia/reperfusion injury in rabbit heart [26].

NS1619, an opener of the mitoK_{Ca} channel has cardioprotective effect in guinea pig, and protection is blocked by the blocker paxilline [44,45]. Our recent study has reported that there was no cross-talk between mitoK_{ATP} and mitoK_{Ca} – that is, paxilline blocked effects of NS1619 but not diazoxide, and 5-hydroxydecanoate blocked effects of diazoxide but not NS1619 [26]. Cao et al. observed similar absence of cross-talk in cardioprotection experiments [45]. These findings suggest distinct channels with distinct pharmacology and suggest that these two channels constitute alternative mechanisms for raising matrix K^+ and generating ROS.

Large-conductance Ca^{2+}-activated K^+ channels are ubiquitously expressed in both excitable and non-excitable cells and contribute to diverse physiological processes. Large-conductance Ca^{2+}-activated K^+ channels are composed of tetrameric sets consisting of a pore-forming α-subunit and an auxiliary subunit, β-subunit [46]. In contrast to ATP-sensitive K^+ channels, the large-conductance Ca^{2+}-activated K^+ channels are not functionally expressed in the

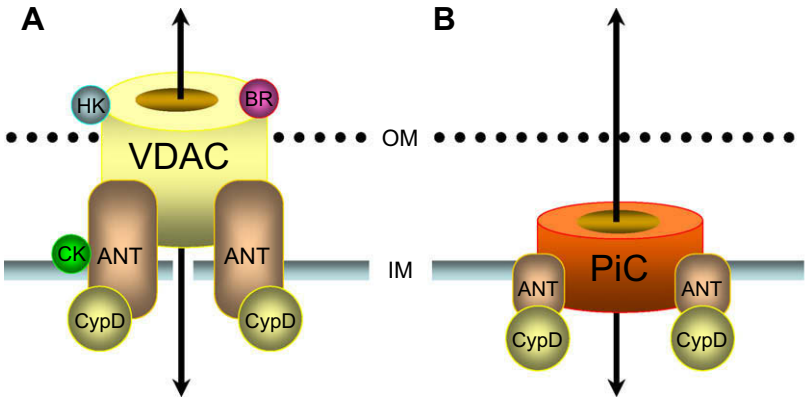

Fig. 1. (A) The putative molecular components of mitochondrial permeability transition pore (mPTP) formation. As described in the text, the core components of mPTP have been thought to be cyclophilin D (CyPD), the adenine nucleotide translocase (ANT) and the voltage-dependent anion channel (VDAC, also known as porin). In addition, benzodiazepine receptor (BR), creatinekinase (CK), and hexokinase (HK) have been suggested to play a regulatory role although the evidence for this is very modest. (B) Revised mechanism of mPTP in light of recent findings in gene-targeted mice. VDAC is no longer part of the model and it appears that an outer membrane component may not even be necessary for this process. ANT now appears to be more of a regulatory protein, and only CypD remains as an established component. The mitochondrial phosphate carrier (PiC) has been added to model as a potential candidate for the pore-forming unit. IM, inner mitochondrial membrane, OM, outer mitochondrial membrane.

sarcolemma of cardiomyocytes. However, some studies [44] has suggested that a large-conductance Ca^{2+}-activated K^+ channel is expressed in the mitochondrial inner membrane of ventricular myocytes and of human glioma cells. Studies of the pharmacological and biochemical profiles have proposed indeed that mitoK_{Ca} channels belong to the Slo-1 family [43,44]; however, the molecular components of mitoK_{Ca} channels also have not been identified as mitoK_{ATP} channels. Recent study has shown that large-conductance Ca^{2+}-activated K^+ channel β1 subunit transcripts are expressed in mammalian cardiac mitochondria [47]. Identification of functional molecules forming mitochondrial K^+ channels may make considerable progress in the search for a new type of cardioprotective agents.

4. Mitochondrial permeability transition pore

Under normal physiological conditions, the mitochondrial inner membrane is impermeable to all but a few selected metabolites and ions; this is essential to maintain the membrane potential and pH gradient that together drive ATP synthesis through oxidative phosphorylation. However, under conditions of high matrix Ca^{2+}, especially when this is accompanied by oxidative stress, high phosphate and low adenine nucleotide concentrations, a non-specific pore opens in the inner mitochondrial membrane known as the mPTP. The mitochondrial permeability transition, first detected as a large amplitude swelling in response to a variety of effectors (Ca^{2+}, phosphate, and ROS and so on), has been known from the 1950s [48]. The mPTP is a large-conductance (~1 nS) mitochondrial megachannel that allows the passage of solutes with molecular weights up to ~1.5 kDa non-selectively. Therefore, opening of this channel has two major consequences. First, it allows unrestricted proton movement across the inner membrane, causing oxidative phosphorylation to be uncoupled. Not only does this prevent ATP synthesis but it also enables the proton-translocating ATPase to reverse direction and so actively hydrolyse ATP rather than synthesise it. Under such conditions, intracellular ATP concentrations rapidly decline, leading to the disruption of ionic and metabolic homeostasis and activation of degradative enzymes such as phospholipases, nucleases and proteases [49]. Unless pore closure occurs, these changes will cause irreversible damage to the cell. Secondly, opening of just a single pore in one mitochondrion is likely to cause its immediate depolarization of $\Delta\Psi m$. This will then activate further pore opening in the same mitochondrion since mPTP opening of calcium loaded mitochondria is activated by depolarization of $\Delta\Psi m$ [50]. Thus mitochondria are either fully open or closed, and it is the fully open state that leads to the mitochondria swelling [51]. Opening of the mPTP is promoted by high matrix Ca^{2+}, inorganic phosphate, ROS, fatty acids, and depolarization in $\Delta\Psi m$, which all occur during ischemia/reperfusion. Mg^{2+}, adenine nucleotides, low pH, and cyclosporine A inhibit mPTP opening.

Based upon biochemical and pharmacological studies, the pore was proposed to consist of the voltage-dependent anion channel (VDAC) in the outer membrane, the adenine nucleotide translocase (ANT) in the inner membrane, plus cyclophilin D (CypD) which mediates the Ca^{2+} sensitivity of this channel in the matrix [49]. VDAC, ANT, and CypD interact at membrane contact sites and reconstitution of this complex in vesicles yields a Ca^{2+}-sensitive channel reminiscent of the mPTP [52].

Prior to the identification of VDAC, researchers assumed that the outer mitochondrial membrane was permeable to small metabolites and solutes. In lipid bilayers, VDAC can be gated by voltage, as implied by its name. Voltage can alter the permeability of the channel and its selectivity for anions versus cations [53]. The open state, which occurs at low voltage (~10 mV, either positive or negative), is a high-conductance state that allows transport of metabolites such as ATP and has weak selectivity for anions over cations. The closed state, which predominates at higher voltages (more than 30 mV, either positive or negative), is a low-conductance state for anions, with very low transport of adenine nucleotides and high permeability for cations.

VDAC has always been considered a key component of the mPTP. This was based on an original hypothesis by Zoratti et al. [54,55], who suggested that the electrophysiological properties of the mPTP were reminiscent of those of the VDAC channel. However, the recent demonstration that mitochondria from VDAC1 knockout mice possess a normal PTP has questioned this proposal [56]. Similarly, Baines et al. have reported that Ca^{2+}-induced mPTP was unaffected in either VDAC1$^{-/-}$ or VDAC3$^{-/-}$ mitochondria [57]. These data indicate that VDAC is not an obligatory member of the PTP protein complex. Moreover, the PTP was still present in mitochondria from livers of mice lacking all three isoforms of ANT [58]. Therefore, with the exception of CypD, confirmation of an obligatory role of these proteins in mPTP is lacking. As an alternative to this pore-forming unit, He and Lemasters have proposed that the pore itself is actually formed from any misfolded inner membrane proteins that would be generated during a toxic stress, and that

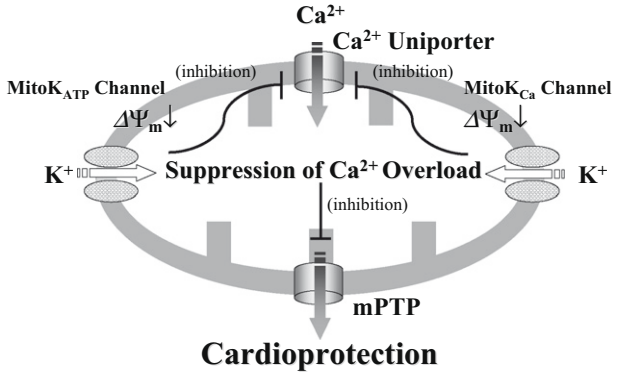

Fig. 2. Schematic representation of the mechanism of protection mediated by mitochondrial ion channels. Activation of two protective mitochondrial K^+ channels, mitochondrial ATP-sensitive K^+ ($mitoK_{ATP}$) channel and mitochondrial Ca^{2+}-activated K^+ ($mitoK_{Ca}$) channels, depolarizes mitochondrial membrane potential ($\Delta\Psi m$), which reduces the driving force for Ca^{2+} influx through mitochondrial Ca^{2+} uniporter, thereby attenuating the mitochondrial Ca^{2+} overload. Consequently, prevention of matrix Ca^{2+} overload inhibits the opening of mitochondrial permeability transition pore (mPTP) and protects heart cell death by antiapoptic mechanism.

CypD acts a regulator through its chaperone-like ability to bind to such denatured proteins [59]. Recently, mitochondrial phosphate carrier has been added to model as a potential candidate for the pore-forming unit of the mPTP (Fig. 1) [60].

There is considerable evidence that the mPTP opens during ischemia/reperfusion, and it has been linked to cytochrome c release and the triggering of apoptosis [61]. Griffiths et al. reported that the mPTP does not open during ischemia but is activated upon reperfusion [62]. Since the mPTP opens when there is significant Ca^{2+} overload and severe energy depletion, and it mediates loss of matrix constituents, mPTP activation likely represents an irreversible terminal event for the mitochondria. We have postulated that mitochondrial K^+ channel activation is involved in cardioprotection and anti-apoptosis through the inhibition of mPTP.

5. Concluding remarks

Recent years have brought robust advances in our understanding of mitochondrial ion channels and the pivotal role of mitochondria in cardioprotection against ischemia/reperfusion injury. In addition, novel imaging and patch-clamp techniques are now being applied to identify and characterize mitochondrial ion channels and the agents that modulate them.

Four different ion channels on mitochondrial inner or outer membrane have been reported to contribute to the cytoprotection in this review article: the $mitoK_{ATP}$ channel, the $mitoK_{Ca}$ channel, the mitochondrial Ca^{2+} uniporter, and the mPTP. Our current outstanding about the link between mitochondrial ion channels and cardioprotection against ischemia/reperfusion injury is summarized in Fig. 2. Unfortunately, the molecular identity of these channels remains unknown yet. In order to truly understand the mechanisms that mediate mitochondrial-driven cell death, it is urgent that we identify the pore-forming units of these channels. It is therefore essential that we continue to screen for novel pore candidates using a variety of genetic and pharmacological technologies. The pharmacological and structural profile of these channels is of paramount importance, since targeting these channels may provide new therapeutic strategies for ischemic heart disease.

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References

[1] Garlid, K.D. (1996) Cation transport in mitochondria – the potassium cycle. Biochim. Biophys. Acta 1275, 123–126.
[2] O'Rourke, B. (2004) Evidence for mitochondrial K^+ channels and their role in cardioprotection. Circ. Res. 94, 420–432.
[3] Halestrap, A.P. (1989) The regulation of the matrix volume of mammalian mitochondria in vivo and in vitro and its role in the control of mitochondrial metabolism. Biochim. Biophys. Acta 973, 355–382.
[4] Juhaszova, M., Zorov, D.B., Kim, S.H., Pepe, S., Fu, Q., Fishbein, K.W., ZIman, B.D., Wang, S., Ytrehus, K., Antos, C.L., Olson, E.N. and Sollott, S.J. (2004) Glycogen synthase kinase-3beta mediates convergence of protection signaling to inhibit the mitochondrial permeability transition pore. J. Clin. Invest. 113, 1535–1549.
[5] Jennings, R.B. and Ganote, C.E. (1976) Mitochondrial structure and function in acutemyocardial ischemic injury. Circ. Res. 38, I80–91.
[6] Turrens, J.F. (2003) Mitochondrial formation of reactive oxygen species. J. Physiol. 552, 335–344.
[7] Costa, A.D., Jakob, R., Costa, C.L., Andrukhiv, K., West, I.C. and Garlid, K.D. (2006) The mechanism by which $mitoK_{ATP}$ opening and H_2O_2 inhibit the mitochondrial permeability transition. J. Biol. Chem. 281, 20801–20808.
[8] Vainio, H., Mela, L. and Chance, B. (1970) Energy dependent bivalent cation translocation in rat liver mitochondria. Eur. J. Biochem. 12, 387–391.
[9] Reed, K.C. and Bygrave, F.L. (1974) The inhibition of mitochondrial calcium transport by lanthanides and ruthenium red. Biochem. J. 140, 143–155.
[10] Gunter, T.E. and Pfeiffer, D.R. (1990) Mechanisms by which mitochondria transport calcium. Am. J. Physiol. 258, C755–C786.
[11] Denton, R.M. and McCormack, J.G. (1990) Ca^{2+} as a second messenger within mitochondria of the heart and other tissues. Annu. Rev. Physiol. 52, 451–466.
[12] Balaban, R.S., Bose, S., French, S.A. and Territo, P.R. (2003) Role of calcium in metabolic signaling between cardiac sarcoplasmic reticulum and mitochondria in vitro. Am. J. Physiol. Cell Physiol. 284, C285–C293.
[13] Litsky, M.L. and Pfeiffer, D.R. (1997) Regulation of the mitochondrial Ca^{2+} uniporter by external adenine nucleotides: the uniporter behaves like a gated channel which is regulated by nucleotides and divalent cations. Biochemistry 36, 7071–7080.
[14] Montero, M., Lobaton, C.D., Hernandez-Sanmiguel, E., Santodomingo, J., Vay, L., Moreno, A. and Alvarez, J. (2004) Direct activation of the mitochondrial calcium uniporter by natural plant flavonoids. Biochem. J. 384, 19–24.
[15] Kirichok, Y., Krapivinsky, G. and Clapham, D.E. (2004) The mitochondrial calcium uniporter is a highly selective ion channel. Nature 427, 360–364.
[16] Trenker, M., Malli, R., Fertschai, I., Levak-Frank, S. and Graier, W.F. (2007) Uncoupling proteins 2 and 3 are fundamental for mitochondrial Ca^{2+} uniport. Nat. Cell Biol. 9, 445–452.
[17] Brookes, P.S., Parker, N., Buckingham, J.A., Vidal-Puig, A., Halestrap, A.P., Gunter, T.E., Nicholls, D.G., Bernardi, P., LeMasters, J.J. and Brand, M.D. (2008) UCPs – unlikely calcium porters. Nat. Cell Biol. 11, 1235–1237.
[18] Griffiths, E.J., Ocampo, C.J., Savage, J.S., Stern, M.D. and Silverman, H.S. (2000) Protective effects of low and high doses of cyclosporin A against reoxygenation injury in isolated rat cardiomyocytes are associated with differential effects on mitochondrial calcium levels. Cell Calcium 27, 87–95.
[19] Lamping, K.A., Christensen, C.W., Pelc, L.R., Warltier, D.C. and Gross, G.J. (1984) Effects of nicorandil and nifedipine on protection of ischemic myocardium. J. Cardiovasc. Pharmacol. 6, 536–542.
[20] Inoue, I., Nagase, H., Kishi, K. and Higuti, T. (1991) ATP-sensitive K^+ channel in the mitochondrial inner membrane. Nature 352, 244–247.
[21] Paucek, P., Mironova, G., Mahdi, F., Beavis, A.D., Woldegiorgis, G. and Garlid, K.D. (1992) Reconstitution and partial purification of the glibenclamide-sensitive, ATP-dependent K^+ channel from rat liver and beef heart mitochondria. J. Biol. Chem. 267, 26062–26069.
[22] Szewczyk, A., Mikolajek, B., Pikula, S. and Nalecz, M.J. (1993) Potassium channel openers induce mitochondrial matrix volume changes via activation of ATP-sensitive K^+ channel. Pol. J. Pharmacol. 45, 437–443.
[23] Garlid, K.D., Paucek, P., Yarov-Yarovoy, V., Murray, H.N., Darbenzio, R.B., D'Alonzo, A.J., Lodge, N.J., Smith, M.A. and Grover, G.J. (1997) Cardioprotective effect of diazoxide and its interaction with mitochondrial ATP-sensitive K^+ channels: possible mechanism of cardioprotection. Circ. Res. 81, 1072–1082.
[24] Liu, Y., Sato, T., O'Rourke, B. and Marbán, E. (1998) Mitochondrial ATP-dependent potassium channels: novel effectors of cardioprotection? Circulation 97, 2463–2469.
[25] Ishida, H., Hirota, Y., Genka, C., Nakazawa, H., Nakaya, H. and Sato, T. (2001) Opening of mitochondrial K_{ATP} channels attenuates the ouabain-induced calcium overload in mitochondria. Circ. Res. 89, 856–858.
[26] Sato, T., Saito, T., Saegusa, N. and Nakaya, H. (2005) Mitochondrial Ca^{2+}-activated K^+ channels in cardiac myocytes: a mechanism of the cardioprotective effect and modulation by protein kinase A. Circulation 111, 198–203.
[27] Bajgar, R., Seetharaman, S., Kowaltowski, A.J., Garlid, K.D. and Paucek, P. (2001) Identification and properties of a novel intracellular (mitochondrial) ATP-sensitive potassium channel in brain. J. Biol. Chem. 276, 33369–33374.

[28] Jezek, P., Orosz, D.E. and Garlid, K.D. (1990) Reconstitution of the uncoupling protein of brown adipose tissue mitochondria. Demonstration of GDP-sensitive halide anion uniport. J. Biol. Chem. 265, 19296–19302.
[29] Jaburek, M., Yarov-Yarovoy, V., Paucek, P. and Garlid, K.D. (1998) State-dependent inhibition of the mitochondrial K_{ATP} channel by glyburide and 5-hydroxydecanoate. J. Biol. Chem. 273, 13578–13582.
[30] Nishida, H., Sato, T., Miyazaki, M. and Nakaya, H. (2008) Infarct size limitation by adrenomedullin: protein kinase A but not PI3-kinase is linked to mitochondrial K_{Ca} channels. Cardiovasc. Res. 77, 398–405.
[31] Hassinen, I. and Chance, B. (1968) Oxidation-reduction properties of the mitochondrial flavoprotein chain. Biochem. Biophys. Res. Commun. 31, 895–900.
[32] Sato, T., Sasaki, N., Seharaseyon, J., O'Rourke, B. and Marban, E. (2000) Selective pharmacological agents implicate mitochondrial but not sarcolemmal K(ATP) channels in ischemic cardioprotection. Circulation 101, 2418–2423.
[33] Grover, G.J. and Garlid, K.D. (2000) ATP-sensitive potassium channels: a review of their cardioprotective pharmacology. J. Mol. Cell Cardiol. 32, 677–695.
[34] Seino, S. (1999) ATP-sensitive potassium channels: a model of heteromultimeric potassium channel/receptor assemblies. Annu. Rev. Physiol. 61, 337–362.
[35] Inagaki, N., Gonoi, T., Clement, J.P., Wang, C.Z., Aguilar-Bryan, L., Bryan, J. and Seino, S. (1996) A family of sulfonylurea receptors determines the pharmacological properties of ATP-sensitive K^+ channels. Neuron 16, 1011–1017.
[36] Suzuki, M., Li, R.A., Miki, T., Uemura, H., Sakamoto, N., Ohmoto-Sekine, Y., Tamagawa, M., Ogura, T., Seino, S., Marbán, E. and Nakaya, H. (2001) Functional roles of cardiac and vascular ATP-sensitive potassium channels clarified by Kir6.2-knockout mice. Circ. Res. 88, 570–577.
[37] Seharaseyon, J., Ohler, A., Sasaki, N., Fraser, H., Sato, T., Johns, D.C., O'Rourke, B. and Marbán, E. (2000) Molecular composition of mitochondrial ATP-sensitive potassium channels probed by viral gene transfer. J. Mol. Cell Cardiol. 32, 1923–1930.
[38] Miki, T., Suzuki, M., Shibasaki, T., Uemura, H., Sato, T., Yamaguchi, K., Koseki, H., Iwanaga, T., Nakaya, H. and Seino, S. (2002) Mouse model of Prinzmetal angina by disruption of the inward rectifier Kir6.1. Nat. Med. 8, 466–472.
[39] Suzuki, M., Sasaki, N., Miki, T., Sakamoto, N., Ohmoto-Sekine, Y., Tamagawa, M., Seino, S., Marbán, E. and Nakaya, H. (2002) Role of sarcolemmal K_{ATP} channels in cardioprotection against ischemia/reperfusion injury in mice. J. Clin. Invest. 109, 509–516.
[40] Paliwal, R., Costa, G. and Diwan, J.J. (1992) Purification and patch clamp analysis of a 40-pS channel from rat liver mitochondria. Biochemistry 31, 2223–2229.
[41] Singh, H., Hudman, D., Lawrence, C.L., Rainbow, R.D., Lodwick, D. and Norman, R.I. (2003) Distribution of Kir6.0 and SUR2 ATP-sensitive potassium channel subunits in isolated ventricular myocytes. J. Mol. Cell Cardiol. 35, 445–459.
[42] Ardehali, H., Chen, Z., Ko, Y., Mejia-Alvarez, R. and Marban, E. (2004) Multiprotein complex containing succinate dehydrogenase confers mitochondrial ATP-sensitive K^+ channel activity. Proc. Natl. Acad. Sci. USA 101, 11880–11885.
[43] Siemen, D., Loupatatzis, C., Borecky, J., Gulbins, E. and Lang, F. (1999) Ca^{2+}-activated K^+ channel of the BK-type in the inner mitochondrial membrane of a human glioma cell line. Biochem. Biophys. Res. Commun. 257, 549–554.
[44] Xu, W., Liu, Y., Wang, S., McDonald, T., Van Eyk, J.E., Sidor, A. and O'Rourke, B. (2002) Cytoprotective role of Ca^{2+}-activated K^+ channels in the cardiac inner mitochondrial membrane. Science 298, 1029–1033.
[45] Cao, C.M., Xia, Q., Gao, Q., Chen, M. and Wong, T.M. (2005) Calcium-activated potassium channel triggers cardioprotection of ischemic preconditioning. J. Pharmacol. Exp. Ther. 312, 644–650.
[46] Wallner, M., Meera, P. and Toro, L. (1996) Determinant for beta-subunit regulation in high-conductance voltage-activated and Ca^{2+}-sensitive K^+ channels: an additional transmembrane region at the N terminus. Proc. Natl. Acad. Sci. USA 93, 14922–14927.
[47] Ohya, S., Kuwata, Y., Sakamoto, K., Muraki, K. and Imaizumi, Y. (2005) Cardioprotective effects of estradiol include the activation of large-conductance Ca^{2+}-activated K^+ channels in cardiac mitochondria. Am. J. Physiol. Heart Circ. Physiol. 289, H1635–H1642.
[48] Cooper, C. and Tapley, D.F. (1956) The effect of thyroxine and related compounds on oxidative phosphorylation. J. Biol. Chem. 222, 341–349.
[49] Halestrap, A.P., Clarke, S.J. and Javadov, S.A. (2004) Mitochondrial permeability transition pore opening during myocardial reperfusion – a target for cardioprotection. Cardiovasc. Res. 61, 372–385.
[50] Scorrano, L., Petronilli, V. and Bernardi, P. (1997) On the voltage dependence of the mitochondrial permeability transition pore – a critical appraisal. J. Biol. Chem. 272, 12295–12299.
[51] Bernardi, P. (1999) Mitochondrial transport of cations: channels, exchangers, and permeability transition. Physiol. Rev. 79, 1127–1155.
[52] Crompton, M., Virji, S. and Ward, J.M. (1998) Cyclophilin-D binds strongly to complexes of the voltage-dependent anion channel and the adenine nucleotide translocase to form the permeability transition pore. Eur. J. Biochem. 258, 729–735.
[53] Blachly-Dyson, E. and Forte, M. (2001) VDAC channels. IUBMB Life 52, 113–118.
[54] Szabó, I. and Zoratti, M. (1993) The mitochondrial permeability transition pore may comprise VDAC molecules. I. Binary structure and voltage dependence of the pore. FEBS Lett. 330, 201–205.
[55] Szabó, I., De Pinto, V. and Zoratti, M. (1993) The mitochondrial permeability transition pore may comprise VDAC molecules. II. The electrophysiological properties of VDAC are compatible with those of the mitochondrial megachannel. FEBS Lett. 330, 206–210.
[56] Krauskopf, A., Eriksson, O., Craigen, W.J., Forte, M.A. and Bernardi, P. (2006) Properties of the permeability transition in $VDAC1^{-/-}$ mitochondria. Biochim. Biophys. Acta 1757, 590–595.
[57] Baines, C.P., Kaiser, R.A., Sheiko, T., Craigen, W.J. and Molkentin, J.D. (2007) Voltage-dependent anion channels are dispensable for mitochondrial-dependent cell death. Nat. Cell Biol. 9, 550–555.
[58] Kokoszka, J.E., Waymire, K.G., Levy, S.E., Sligh, J.E., Cai, J., Jones, D.P., MacGregor, G.R. and Wallace, D.C. (2004) The ADP/ATP translocator is not essential for the mitochondrial permeability transition pore. Nature 427, 461–465.
[59] He, L. and Lemasters, J.J. (2002) Regulated and unregulated mitochondrial permeability transition pores: a new paradigm of pore structure and function? FEBS Lett. 512, 1–7.
[60] Baines, C.P. (2009) The molecular composition of the mitochondrial permeability transition pore. J. Mol. Cell Cardiol. 46, 850–857.
[61] Crompton, M. (1999) The mitochondrial permeability transition pore and its role in cell death. Biochem. J. 341, 233–249.
[62] Griffiths, E.J. and Halestrap, A.P. (1995) Mitochondrial non-specific pores remain closed during cardiac ischaemia, but open upon reperfusion. Biochem. J. 307, 93–98.